**Substance Group:** **Nitric Acid, 2-Ethylhexyl Ester**

**Summary prepared by:** **Petroleum Additives Panel**
**Health, Environmental and Regulatory Task Group**

# 1.0 Physical/Chemical Characteristics

## 1.1 Partition Coefficient

**Robust Summary 18 – LogKow – 1**

| ***Test Substance*** | |
|---|---|
| CAS # | 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| ***Method*** | |
| Method/Guideline followed | OECD Guideline No. 117 for Testing of Chemicals |
| Test Type | Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method |
| GLP | Yes |
| Year (Study Performed) | 2005 |
| Log $P_{ow}$ Reference Standards | Benzene, Purity: 99.92%, 5.04 x $10^3$ mg/L, Guideline reported log $P_{ow}$ = 2.1<br>Toluene, Purity: >99%, 2.99 x $10^3$ mg/L, Guideline reported log $P_{ow}$ = 2.7<br>Naphthalene, Purity:≥99%, 403 mg/L, Guideline reported log $P_{ow}$ = 3.6<br>Phenanthrene, Purity:≥97%, 31.0 mg/L, Guideline reported log $P_{ow}$ = 4.5<br>Triphenylamine, Purity:≥98%, 214 mg/L, Guideline reported log $P_{ow}$ = 5.7<br>DDT, Purity: 98%, 213 mg/L, Guideline reported log $P_{ow}$ = 6.2 |
| Dead Time Reference Standard | Thiourea, >99% 26 mg/L, mean retention time 1.924 |
| Test Material Solvent | methanol: water (75:25 v/v) |
| Calculations | Partition coefficient was determined from the capacity factor k:<br><br>$K = t_R - t_0/t_0$ where $t_R$ was the retention time of the test substance and $t_0$ was the dead time, the average time the unretained reference component needed to pass through the HPLC column.<br><br>Factor k of the test substance was correlated to its $\log_{10} P_{ow}$ value using the calibration curve obtained for the reference substances. |
| HPLC Conditions | Mobil Phase: methanol: water (75:25 v/v) (ph 7.1)<br>Injection: 25 ul<br>Column: Prodigy $C_{18}$ (150 mm x 4.6 mm)<br>UV Detector Wavelength: 240 nm<br>Detector: Waters 2487 Dual Absorbance Detector<br>Waters 2410 Differential Refractometer<br>Flow Rate: 1.0 mL/minute<br>Column Temperature: $40^OC$ |
| ***Results*** | The test substance (0.0614 g) was diluted to 100 mL with the mobile phase. The test substance, thiourea and reference standards were injected in duplicate. The mean retention times and $Log_{10} P_{ow}$ values of the reference materials and test substance were as follows: |

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| | Mean Retention Time (min) | $Log_{10}$ $P_{ow}$ |
|---|---|---|
| Thiourea | 1.924 | - |
| Benzene | 3.420 | 2.1 |
| Toluene | 4.240 | 2.7 |
| Naphthalene | 4.749 | 3.6 |
| Phenanthrene | 6.975 | 4.5 |
| Triphenylamine | 15.694 | 5.7 |
| DDT | 23.843 | 6.2 |
| Test Substance | 11.378 | 5.24 |

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| ***Conclusions*** | $Log_{10}$ $P_{ow}$ = 5.24<br>Partition Coefficient: 1.73 x $10^5$ |
| ***Data Quality*** | Reliable without restriction. |
| ***References*** | Woolley & Mullee (2005). "Determination of Partition Coefficient." SafePharm Laboratories Report No.: 1666/045. 12 July 2005. |
| ***Other*** | Updated: 8/09/2005 |

# 2.0 Environmental Fate and Pathways

## 2.1 Hydrolysis

**Robust Summary 18-Hydro-1**

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| CAS No. | CAS# 27247-96-7 |
| Test Substance Name | Nitric acid, 2-ethylhexyl ester |
| Method/Guideline | EC Method C7 |
| GLP | Yes |
| Year | 1998 |
| Remarks for Test Conditions | The test substance was dissolved in aqueous media buffered at pH 4, 7 and 9. The concentration of the test substance was determined as a function of time. The logarithms of the concentrations are plotted against time and the slope of the line used to calculate the rate constant. The concentrations in the test solution were determined by a gas chromatographic method using mass spectrometric detection.<br><br>The test substance was used as the analytical standard. Buffer solutions included: pH 4 – phthalate buffer; pH 7 – phosphate buffer; pH 9 – borate buffer. Buffer solutions were incubated in the water bath at the test temperature and their pH adjusted to the nominal value. The test solution was prepared by adding the test material in methanol to the buffer solution to give a final test material concentration of 6 mg/L and a final methanol concentration of approximately 1%.<br><br>The study was conducted in borosilicate glass bottles with minimal headspace closed with Teflon lined screw caps.<br><br>The study was conducted in two phases as follows:<br>Phase I – the test material solution prepared at pH 4, 7 and 9 at 6 mg/L and incubated at 50 $^{o}$C. Analysis conducted over several time points. Results obtained at 2.4 hours and 5 days (extrapolated) were used to determine if additional testing was necessary.<br>Phase II - the test material solution prepared at pH 4, 7 and 9 at 6 mg/L and incubated at 25 $^{o}$C. Analysis conducted over several time points. This test was run in duplicate.<br><br>The logs of the concentration of the test material in the different buffers incubated at 50 $^{o}$C were plotted against incubation time and fitted to linear regression curves with good coefficients of correlation for each pH. The observed rate constant of the reaction was calculated from the slope for each pH ($k_{obs}$ = slope x 2.303). Reaction half-life was calculated ($t_{1/2} = 0.693/k_{obs}$). |

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| Results | The observed rate constants and reaction half lives at 50 and 25 $^{o}$C were as follows:<br><br>pH: 4.0 — Rate Constant (min$^{-1}$) (50 $^{o}$C): 0.0005657 — Half Life (mins) (50 $^{o}$C): 1225 — Rate Constant (hour $^{-1}$) (25 $^{o}$C): 0.001874, 0.002875 — Half Life (hours) (25 $^{o}$C): 370, 241<br>pH: 7.0 — Rate Constant (min$^{-1}$) (50 $^{o}$C): 0.0004698 — Half Life (mins) (50 $^{o}$C): 1475 — Rate Constant (hour $^{-1}$) (25 $^{o}$C): 0.004405, 0.008664 — Half Life (hours) (25 $^{o}$C): 157, 80<br>pH: 9.0 — Rate Constant (min$^{-1}$) (50 $^{o}$C): 0.0004072 — Half Life (mins) (50 $^{o}$C): 1702 — Rate Constant (hour $^{-1}$) (25 $^{o}$C): 0.004695, 0.006441 — Half Life (hours) (25 $^{o}$C): 148, 108<br><br>The duplicate value at pH 7, 25 $^{o}$C was excluded.<br><br>The test material was shown to hydrolyze in each of the pH conditions tested following a pseudo-first order reaction. The mean half-life of the hydrolysis reaction at 25$^{o}$C ranged from 370 (pH 4.0) to 108 hours (pH 9.0). |
| Conclusions | The test material was shown to hydrolyze in each of the pH conditions tested following a pseudo-first order reaction. The mean half-life of the compound and water reaction at 25$^{o}$C ranged from 370 hours (pH 4.0) to 108 hours (pH 9.0). |
| Data Quality | Reliable without restriction (Klimisch Code) |
| References | Confidential Business Information |
| *Other* | Updated: 11/11/2003 |
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## 2.2 Biodegradation

**Robust Summary 18-Biodeg – 1**

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|---|---|
| ***Test Substance*** | |
| CAS # | 27247-96-7 |
| Chemical Name | Nitric acid, 2 ethylhexyl ester |
| ***Method*** | |
| Method/Guideline Followed | Ready Biodegradability, $CO_2$ Headspace Test (ISO Guideline No. (14593) |
| Test Type (aerobic/anaerobic) | Aerobic |
| GLP (Y/N) | Y |
| Year (study performed) | 2005 |
| Contact time (units) | 28 days |
| Test apparatus | 107 mL of inoculated culture medium added to each of 25 replicate test vessels which were then sealed using Teflon lined silicon septa and aluminum crimp caps. After sealing 4.0 µl of test material was injected through the septum of each vessel to give test material concentration of 36.5 mg/L (20 mg carbon/L). 29 control vessels prepared as above without the addition of the test material. |
| Inoculum | Sewage sludge microorganisms from a domestic wastewater treatment plant. Sample filtered through coarse filter paper, filtrate purged with $CO_2$ free air for approximately 1 hour while maintaining pH at 6.5 using concentrated orthophosphoric acid. The pH was then adjusted to 7.6 with sodium hydroxide and the inoculum allowed to settle for approximately 1 hour prior to the removal of the supernatant for use in the study. |
| Replicates: | Duplicates for the control, standard material, test material and toxicity control at each sampling point (5 replicates were available for day 28 analysis). Headspace to liquid ratio was 1:2. |
| Temperature of incubation: | 20±1 °C |
| Study initiation: | Test cultures provided with $CO_2$ free air and placed on a rotary shaker and mixed at 150 rpm for the study duration. |
| Sampling: | Days 2, 6, 8, 10, 14, 16, 20, 22, 24, 28 (after acidification on day 28) |
| Concentration of test substance: | 36.5 mg/L (20 mg carbon/L) |
| Controls: | Toxicity, blank and positive controls used per guideline. Positive control was sodium benzoate added to the control vessel at a loading of 20 mg C/L. Both test substance and reference material were added to the toxicity controls to obtain a maximum concentration of 40 mg C/L |
| Analytical method: | Headspace samples were analyzed for inorganic carbon using a Total Organic Carbon analyzer. Analysis was carried out in triplicate. Dissolved organic carbon analysis was not possible due to the insoluble nature of the test material in water. |
| Study termination: | An aliquot of concentrated orthophosphoric acid was injected through the septum of each vessel taken for analysis in order to reduce pH to <3. The vessels were then shaken at ~150 rpm for 1 hour at 20±1 °C prior to samples being withdrawn from the headspace for analysis. |
| Method of calculating biodegradation values: | Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material. |

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| ***Results*** | The test substance was not considered readily biodegradable under the criteria that requires 60% biodegradation within 28 days, achieved within 10 days of reaching 10% biodegradation. The $CO_2$ production from the reference chemical exceeded the 60% of theoretical necessary to consider the test valid. The degradation for the toxicity control was 34% as of day 28 therefore the test substance was not considered to be inhibitory at the concentration tested. The water solubility of this test material is relatively low and coupled with the volatile nature of the test material, this may have resulted in the majority of the test material being present in the headspace and not proportionally dissolving in the test media. The test material may not have been bioavailable and this may have contributed to the lack of biodegradation. |
| ***Degradation % After Time*** | Test substance: 0 % degradation in 28days<br>Positive control substance: 76% in 28 days |
| ***Conclusions*** | The test substance was not readily biodegradable under the conditions of this study.. |
| ***Data Quality*** | (1) Reliable without restriction |
| ***References*** | Clarke, N. "Assessment of Ready Biodegradability; CO2 in Sealed Vessels (CO2 Headspace). SafePharm Project Number 1666/047. 23 September 2005. |
| ***Other*** | Updated: 11/11/2005 |

## 2.3 Photodegradation

**Robust Summary 18-Photodeg – 1**

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|---|---|
| ***Test Substance*** | |
| CAS # | 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| ***Method*** | |
| Method/Guideline followed | OECD Guideline No. 101 for Testing of Chemicals |
| Test Type | Determination of Photochemical Degradation |
| GLP | Yes |
| Year (Study Performed) | 2005 |
| Test Material Solvent | Methanol |
| Calculations | The molar absorption coefficient was calculated based on absorbance and concentration as follows:<br><br>Molar absorption coefficient = A/cl where A = absorbance at 290 nm, c = concentration ($mol.dm^{-3}$), and l = cell path length (1 cm).<br><br>Concentration ($mol.dm^{-3}$) = concentration (g/L)/ molecular weight ($g.mol^{-1}$)<br><br>The molarity of the test solution was calculated using a molecular weight of 175.2 $g.mol^{-1}$. |
| Absorption Determination | Absorption was determined using a Perkin-Elmer Lambda 20 double-beam spectrophotometer over a wavelength range of 200 to 800 nm using a quartz cell with a 1 cm cell path length. |
| ***Results*** | The test substance was found to have a molar absorption coefficient of 6.07 $dm^{-3}.mol.cm^{-1}$ and an absorbance of 0.0096113 at 290 nm. The concentration of the test substance was $1.581 \times 10^{-3}$ $mol.dm^{-3}$. It was clear from the absorption spectrum that the test material did not exhibit any significant absorbance at wavelengths greater than 250 nm. In order to assess if a photochemical degradation study is required the test substance must have significant absorbance at wavelengths greater than 290 nm. The critical value for the study to be performed requires a molar absorption coefficient to be >10 at 290 nm.<br><br>This test substance had a molar absorption coefficient of <10 at 290 nm. Therefore no photochemical degradation study was required. |
| *Conclusions* | This test substance had a molar absorption coefficient of <10 at 290 nm. Therefore a photochemical degradation study was not required. |
| *Data Quality* | Reliable without restriction. |
| *References* | Mullee, D. "Determination of Photochemical Degradation." SafePharm Laboratories Report No.: 1666/046. 11 July 2005. |
| *Other* | Updated: 8/09/2005 |

## 3.0. Ecotoxicity

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## 3.1 Acute Toxicity to Fish

**Robust Summary 18-Fish –1**

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|---|---|
| Test Substance | |
| CAS # | 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| Method | |
| Method/Guideline followed | OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1992). |
| Test Type | Acute Toxicity to Fish (Static test) |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1998 |
| Species/Strain | Zebra fish (Danio rerio) |
| Fish Number | 10/concentration |
| Fish Size | Average length 2.67 cm (2.4-3.0 cm) ; Biological loading 0.3 g/L |
| Analytical Monitoring | Not performed |
| Nominal Test Substance Concentration Levels | Control, 1, 10 and 12.6 mg/L (12.6 mg/L is the limit of solubility) |
| Test Concentration Preparation | Appropriate amounts of the test material were added directly to the experimental water (4 liters). Both the mid and high concentrations were subjected to slight continuous stirring (<20 rpm). |
| Exposure Period | 96 hours |
| Exposure Conditions | Static (non-renewal test) conditions. |
| Vehicle | None |
| Statistical Analysis | None required based on the results. |
| Dose Rangefinding Study | No |
| Test Chambers | 4-liter tank containing 4 liters of test solution |
| Diluent Water | Reverse osmosis tap water |
| Test Solution Water Chemistry During Exposures | Conductivity: 200 umhos/cm<br>Dissolved Oxygen: 100-118% of dissolved air saturation value<br>pH: 6.46-6.83<br>Hardness: 90 mg/L $CaCO_3$ |
| Photoperiod | 12-h light per day |
| Temperature Range | 21.1-22.3$^{O}$C |

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|---|---|
| Positive Control | No |
| Remarks field for test conditions | Pretreatment: none, fish held for a minimum of 12 days before testing. No feeding 48 hours prior to and during the test. All organisms were observed for mortality and clinical signs of toxicity or abnormal behavior at 2, 24, 48, 72, and 96 hours after initiation of test material exposure. |
| Results | Cumulative mortality at study termination (96 hours) was as follows:<br><br>Test Substance Concentration (mg/L) / N / Cumulative Mortality / % Mortality<br>1.0 / 10 / 3 / 30<br>10.0 / 10 / 2 / 20<br>12.6 / 10 / 1 / 10<br><br>No undissolved test material was seen on the surface of the test vessels during the study. |
| Conclusions | The 24, 48, 72 and 96 hour LC50s were >12.6 mg/L. |
| Data Quality | Reliable with restriction, restriction due to the lack of analytical confirmation of dose concentration. |
| References | Unpublished confidential business information |
| Other | Updated: 11/06/2003 |
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## 3.2 Acute Toxicity to Invertebrates (e.g., *Daphnia*)

**Robust Summary 18-Daph-1**

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|---|---|
| ***Test Substance*** | |
| CAS # | 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984), EEC Directive 92/69-Method C.2 (1992). |
| Test Type | Static (non-renewal) acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1998 |
| Species/Strain | *Daphnia magna* |
| Analytical Monitoring | None |
| Exposure Period (unit) | 48 hours |
| Statistical methods | None required based on results. |
| Remarks field for test conditions (fill as applicable) | Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.<br>Appropriate amounts of the test material were added directly to the dilution water and stirred for 10 to 14 minutes.<br>Twenty daphnids, less than 24 hours old were distributed into each concentration (50 mL/chamber)(5 daphnids/replicate). Daphnids were not fed during exposure. Control test chambers were handled in an identical fashion.<br>Light cycles were maintained at 12-hours of light per day. Test solutions were maintained at 19.3-19.9$^{o}$C.<br>Dilution water was prepared according to the guideline and contained 200 mL demineralized water and 800 mL natural water. |
| Test Concentrations | Control, 1, 10 and 12.6 mg/L (12.6 mg/L is the limit of solubility) |
| ***Results*** | |
| Remarks | Water chemistry: Dissolved oxygen: 8.4 – 8.7 mg/L; pH: 7.65 – 7.80<br><br>100% survival occurred in all control, 1 and 10 mg/L test vessels. At 12.6 mg/L 30% immobilization was observed at 24 hours and 20% immobilization was noted at 48 hours. The 24 and 48-hour EC50 values were both >12.6 mg/L. The 24 and 48 hour NOEC was 10 mg/L. |

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| ***Conclusions*** | The 24 and 48-hour EC50s were >12.6 mg/L. The 24 and 48 hour NOEC was 10 mg/L. |
| ***Data Quality*** | Reliable with restriction, restriction due to the lack of analytical confirmation of dose concentration. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/07/2003 |

## 3.2 Acute Toxicity to Algae

**Robust Summary 18-ALG-1**

| | |
|---|---|
| *Test Substance* | |
| CAS # | 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| Method | |
| Method/Guideline followed | OECD Guideline for Testing of Chemicals #201 Alga Growth Inhibition Test (1984); EEC Directive 92/69 Method C.3 (1992) |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1998 |
| Species/Strain | *Selenastrum capricornutum* (CCAP 278/4) |
| Element basis (# of cells/mL) | Approximately 10,000 cells/mL |
| Exposure period/duration | 72 hours |
| Analytical monitoring | None |
| Statistical methods | The area under the growth curve and the average specific growth rate were determined. |
| Remarks field for test conditions (fill as applicable) | Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of test media and stirred for 10 to 18 minutes.<br>A 72-hour sealed static test was carried out in 250 mL Erlenmeyer flasks filled with 50 mL of test solution. Three flasks were prepared for each test concentration and the control. Control flasks containing algal growth medium only. Test chambers were sealed and incubated. The flasks were shaken throughout the study. The pH was determined at time 0 and at 72 hours. Air temperature in the test incubator was monitored throughout the study. Cell counts were made at the start of the study and then at approximately 24-hour intervals. PH was determined for each culture at study start and at approximately 72 hours.<br><br>Test Levels: Control, 1.0, 10 and 12.5 mg/L (limit of solubility). |
| *Results* | The 72-hour No Observed Effect Concentration, based on growth rate and growth inhibition, was 12.6 mg/L, the highest concentration tested. |
| Remarks | The effective initial concentrations, which induce 50% inhibition as, determined by comparison of area under the growth curve and comparison of growth rates were determined. No significant cell growth or growth rate inhibition was recorded during the 72-hour test period up to 12.6 mg/L of test substance. The no observed effect concentration was defined as the concentration of test substance, which induced less than 25% inhibition. |

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| | The 72-hour loading rates which resulted in 50% reduction in culture growth based on areas under the growth curves and average specific growth rates were both >12.6 mg/L.<br><br>The highest No Observed Effect Level was 12.6 mg/L .<br><br>Test Substance Concentration (mg/L) / 72 Hour % Inhibition Cell Growth / 72 Hour % Inhibition Growth Rate<br>0 / - / -<br>1.0 / 20 / 6<br>10.0 / 14 / 2<br>12.6 / 0 / 0<br><br>pH range 7.05-7.20 |
| *Conclusions* | The 72-hour No Observed Effect Concentration, based on growth rate and growth inhibition, was 12.6 mg/L, the highest concentration tested. |
| *Data Quality* | Reliable with restriction, restriction due to the lack of analytical confirmation of dose concentration. |
| *References* | Confidential business information. |
| *Other* | Updated: 10/31/2003 |

## 4.0 Mammalian Toxicity

### 4.1 Acute Toxicity

#### 4.1.1 Acute Oral Toxicity

**Robust Summary 18-Acute Oral -1**

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | FHSA 16CFR1500.3 |
| Test Type | Acute oral toxicity |
| GLP (Y/N) | N |
| Year (Study Performed) | 1978 |
| Species/Strain | Rats/ Sprague-Dawley strain |
| Sex | Male/Female |
| No. of animals/dose | 5/sex |
| Vehicle | None |
| Route of administration | Oral (intragastric) |
| Dose level | 10 mL/kg |
| Control group included | No |
| Remarks field for test conditions | A single dose of the test material was administered intragastrically to five male and five female rats. The animals were observed for signs of toxicity during a 14-day observation period. All surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals after 14 days. |
| ***Results*** | LD50 >10 mL/kg (males and females) |
| Remarks | Two males and one female died during the observation period. No other signs of toxicity were noted. There were no gross lesions observed during necropsy. |
| ***Conclusions*** | The test article, when administered to male and female Sprague-Dawley rats, had an acute oral LD50 of >10 mL/kg. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code). |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 6/05/2003 |
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## 4.1.2 Acute Dermal Toxicity

**Robust Summary 18-Acute Dermal-1**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | Similar to OECD Guideline 402; FHSA Section 191.12 |
| Test Type | Acute dermal toxicity (Limit Test) |
| GLP (Y/N) | N |
| Year (Study Performed) | 1978 |
| Species/Strain | Rabbits/Albino |
| Sex | Not specified |
| No. of animals | 4 |
| Vehicle | None |
| Route of administration | Dermal |
| Dose level | 5 mL/kg |
| Control group included | No |
| Remarks field for test conditions | This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that the skin of all treated animals was abraded prior to dosing. In addition the guideline calls for the evaluation of five males and five females using at least one dose level. This study was conducted using two males and two females. Given the high dose level tested during this study and the lack of any mortality, these deviations were not considered sufficient to disqualify this study.<br><br>Prior to topical application of the test material, the hair on the abdomen of each animal was closely clipped. The skin of all treated animals was abraded prior to dosing. A single dose of 5 mL/kg of the undiluted test material was administered dermally to the abraded skin of all animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a rubber dam. The application site was washed with warm water and wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for 14 days after treatment. |
| ***Results*** | LD50 > 5 mL/kg |
| Remarks | All animals survived the duration of the study. No signs of toxicity were observed. |

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| ***Conclusions*** | The test article, when administered dermally as received to four albino rabbits had an acute dermal LD50 of greater than 5 mL/kg. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the fact that the study design differs from the referenced guideline. However given the high dose level tested (5 mL/kg) and the lack of mortality the study was considered valid and appropriate for review. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 6/05/2003 |
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## 4.2 Genetic Toxicity

**Robust Summary 18-Gentox-1**

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | Similar to OECD Guideline 471 |
| Test Type | Bacterial Reverse Mutation Assay |
| GLP (Y/N) | N |
| Year (Study Performed) | 1978 |
| Test System | *Salmonella typhimurium* |
| Strains Tested | *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 |
| Exposure Method | Plate incorporation |
| Test Substance Doses/concentration levels | 0.1% solution (v/v) in DMSO: 0.001, 0.005, 0.01, 0.05, 0.1ul/plate |
| Metabolic Activation | With and without (0.5 ml of S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats) |
| Vehicle | Dimethylsulfoxide (DMSO) |
| Tester strain, activation status, Positive Controls and concentration level | TA98 +S9 Aflatoxin B1 1.0 ug/plate<br>TA98 -S9 2-nitroflourene 5.0 ug/plate<br>TA100 +S9 Aflatoxin B1 1.0 ug/plate<br>TA100 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate<br>TA1535 +S9 2-aminoanthracene 5.0 ug/plate<br>TA1535 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate<br>TA1537 +S9 2-aminochrysene 1.0 ug/plate<br>TA1537 -S9 9-aminoacridine 100 ug/plate<br>TA1538 +S9 2-aminofluorene 2.0 ug/plate<br>TA1538 -S9 2-nitroflourene 5.0 ug/plate |
| Vehicle Control | Dimethylsulfoxide (DMSO) 100 ul/plate |
| Statistical Analysis | Mean revertant colony count and standard deviation were determined for each dose point. Linear regression analysis was used to compute the best-fit line of dose response. |
| Dose Rangefinding Study | No |
| S9 Optimization Study | Yes |
| Remarks field for test conditions | This study was conducted in 1978, prior to the adoption of OECD Test Guideline 471. In addition to the tester strains used during this study, the OECD Guideline suggests the inclusion of tester strains *E.coli* WP2 uvrA, or WP2 uvrA (pKM101) or *Salmonella typhimurium* TA102. This study included the use of tester strain TA1538. OECD 471 does not incorporate this strain. These deviations from the test guideline are not considered major study deficiencies. |

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| | In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle control, and a positive control. Three plates/dose group/strain/treatment set were evaluated. Test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, and S9 mix (if needed). This was overlaid onto the surface of supplemented Noble's agar in a screw-capped tube. Tubes were mixed and poured over a base plate of Spizzizen's minimal medium. Plates were incubated for 48 hours at 37$^{o}$C. |
| ***Results*** | The test substance was not genotoxic in this assay with or without metabolic activation. |
| Remarks | In this mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. For each strain, the numbers of revertant colonies in negative control plates were within acceptable limits as defined by historical control data for spontaneous revertants. Sterility controls were negative. |
| ***Conclusions*** | Under the conditions of this study, the test material was not mutagenic. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 6/06/2003 |
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**Robust Summary 18-Gentox-2**

| ***Test Substance*** | |
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| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2 ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | OECD Guideline 473 |
| Test Type | *In Vitro* Chromosomal Aberration Assay |
| GLP (Y/N) | Y |
| Year (Study Performed) | 2005 |
| Test System | Human peripheral blood lymphocytes |
| Exposure Method | Dilution |
| Test Substance concentration levels | Experiment 1<br>4 hour treatment, 20 hour harvest without activation: 0*, 6.88, 13.75*, 27.5*, 55*, 82.5, 110 µg/mL<br>4 hour treatment, 20 hour harvest with activation (2% S9): 0*, 13.75, 27.5*, 55*, 82.5, 110*, 165 µg/mL<br><br>Experiment 2<br>24 hour treatment, 0 hour harvest without activation: 0*, 6.88, 13.75*, 27.5*, 55*, 82.5, 110 µg/mL<br>4 hour treatment, 20 hour harvest with activation (1% S9): 0*, 13.75*, 27.5*, 55*, 82.5, 110, 165 µg/mL<br><br>* = Dose levels selected for metaphase analysis. |
| Metabolic Activation | With and without S9 fraction mix of livers of phenobarbitone and beta-naphthoflavone pretreated Sprague Dawley rats |
| Vehicle | DMSO |
| Vehicle and Positive Control concentration levels by activation status | Mitomycin C - non-activated test system positive control (0.2 or 0.4 µg/mL)<br>Cyclophosphamide - activated test system positive control (5 µg/mL)<br>DMSO – solvent control |
| Statistical Analysis | Positive control groups were compared to vehicle control by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test. |
| Preliminary Toxicity Dose Range Finding Assay | Consisted of an evaluation of test article effect on mitotic index. Evaluation performed at 4 hours with and without activation followed by a 20-hour recovery period and following a continuous 24-hour exposure without metabolic activation. Concentrations of test material evaluated ranged from 6.88 to 1760 µg/mL. |
| Remarks field for test conditions | A pretest dose range finding study was conducted at concentrations up to 1760 µg/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. Mitomycin C (positive control) was tested without activation and Cyclophosphamide (positive control) was tested with activation. Prepared cultures were treated with test substance or control material. Two |

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| | experiments were conducted as follows:<br><br>Experiment 1<br>4 hour treatment, 20 hour harvest without activation: 0*, 6.88, 13.75*, 27.5*, 55*, 82.5, 110 µg/mL<br>4 hour treatment, 20 hour harvest with activation (2% S9): 0*, 13.75, 27.5*, 55*, 82.5, 110*, 165 µg/mL<br><br>Experiment 2<br>24 hour treatment, 0 hour harvest without activation: 0*, 6.88, 13.75*, 27.5*, 55*, 82.5, 110 µg/mL<br>4 hour treatment, 20 hour harvest with activation (1% S9): 0*, 13.75*, 27.5*, 55*, 82.5, 110, 165 µg/mL<br><br>* = Dose levels selected for metaphase analysis.<br><br>Two hours prior to harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.1 ug/mL. Slides were prepared using Giemsa stain. Two-slides/treatment group were evaluated. 200 metaphase cells (100 per culture) were scored. Chromosomes were counted for each cell. Chromosome aberrations were recorded. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. A positive response was recorded for a particular treatment if the percentage of cells with aberrations, excluding gaps, markedly exceeded that seen in the concurrent control, either with or without a clear dose response. For modest increases in aberration frequency a dose response relationship was generally required and appropriate statistical tests were applied. |
| ***Results*** | Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes. |
| Remarks | In the pretest toxicity assay a precipitate of the test material was observed in parallel blood free cultures at the end of exposure at ≥880 µg/mL in the 4(20)-hour and 24 hours exposure groups without metabolic activation. In the 4(20)-hour exposure group with metabolic activation precipitate was observed at and above 440µg/mL.<br><br>In exposed cultures metaphase cells were present up to 55 and 110 µg/mL in the 4/20 hour exposure groups in the absence and presence of metabolic activation respectively. The maximum dose with metaphases present in the 24-hour continuous exposure group without activation was 110 µg/mL. The test material induced a dose related reduction in mitotic index and demonstrated a steep toxicity curve in all exposure groups.<br><br>In Experiment 1, there were no scorable dose levels at 82.5 and 165 µg/mL for the without and with metabolic activation exposure groups. The maximum dose level selected for metaphase analysis, based on toxicity data, was 55 µg/mL without activation and 110 µg/mL with metabolic activation. |

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| | The doses selected for the analysis of chromosome aberrations were 13.75, 27.5, 55, and 82.5 µg/mL without activation and 27.5, 55, 110 and 165µg/mL with activation. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant.<br><br>In Experiment 2, there were scorable metaphases present up to 55 µg/mL without or with metabolic activation. The maximum dose level selected for metaphase analysis, based on toxicity data, was 55 µg/mL with and without activation.<br><br>The doses selected for the analysis of chromosome aberrations were 13.75, 27.5, 55, and 82.5 µg/mL with and without activation. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant.<br><br>Positive and vehicle control group responses were as expected. |
| ***Conclusions*** | Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes in the presences and absence of a liver metabolizing system at dose levels that induced acceptable levels of toxicity. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Wright, NP. "Chromosome Aberration Test in Human Lymphocytes *in Vitro*." SafePharm Study 1666/0079. 5 December 2005. |
| ***Other*** | Updated: 12/21/05 |
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## 4.3 Repeated Dose Toxicity

### Robust Summary 18-Repeat Dose-1

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | Similar to OECD 412 |
| Test Type | 14-day inhalation toxicity studies in rats with 2 week recovery periods |
| GLP (Y/N) | Not Specified |
| Year (Study Reported) | 1987 |
| Species | Rat |
| Strain | Sprague-Dawley CD, 8 weeks of age at initiation of treatment |
| Route of administration | inhalation, nose only exposure |
| Duration of exposure | 6 hours/day |
| Doses/concentration levels | 0, 14, 42, 150 ppm (Study I)<br>0 (unexposed), 0 (chamber room air exposed), 4.3, 42, 420 ppm (Study II) |
| Sex | Male |
| Frequency of treatment | 5 days/week for 2 weeks |
| Control and treatment groups | 10 male rats/group (5/group sacrificed after $10^{th}$ exposure; 5/group held for 2 week recovery period) (Study I)<br>10 male rats/group (5/group sacrificed after $10^{th}$ exposure; 5/group held for 2 week recovery period) (Study II) |
| Post exposure recovery period | 2 Weeks |
| Statistical methods | Methods not specified. |
| Dose rangefinding study | No |
| Remarks field for test conditions | Treated animals were exposed to the test material as mixed aerosol and vapor atmospheres generated by passing dry nitrogen through midget impingers containing the test material at the low and intermediate levels and by nebulization at the high level. Heated water baths were used to promote volatilization of the test material in the impingers and heating tape was used to minimize condensation of vapors in the transfer tubes. Vapors or aerosols were mixed with air prior to entry into the 150 L stainless steel exposure chambers. Chamber housed control animals were exposed to room air only. In the second study a control group of 5 unexposed rats was included in order to evaluate the effects of restraint and fasting on hepatic vacuolation. Chamber exposure concentrations were measured by gas chromatography with flame ionization detection at 60-minute intervals. Rats were weighed and observed daily. In |

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| | Study I overnight urine samples were collected from each rat after the 9th exposure. After the 10th exposure, blood samples were collected from each rat for clinical chemistry and hematology analysis. Five rats from each group were sacrificed for pathological evaluation. Select organs were weighed. After the 14-day recovery period the remaining rats were subjected to the same clinical pathology and microscopic evaluations. Select organs were weighed. A range of tissues was examined microscopically. In the second study similar procedures were followed except clinical pathology parameters were not evaluated and pathology examinations were limited to the liver and kidneys only. |
| ***Results*** | |
| Remarks | **Study I**<br>All animals survived until their intended sacrifice. After the 10th exposure, rats from the 150 ppm group exhibited increased hemoglobin and hematocrit values and increased erythrocyte and platelet counts. Mean absolute and relative liver weights were increased compared to controls. Lipid like cytoplasmic inclusions were found in hepatocytes and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. Following the two-week recovery period mean absolute and relative spleen weights were decreased, clinical pathology was normal and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules.<br><br>After ten exposures at 42 ppm mean absolute and relative liver weights were increased compared to controls. Lipid like cytoplasmic inclusions were found in hepatocytes and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. Following the two-week recovery period mean relative spleen weights were decreased, clinical pathology was normal and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules.<br><br>After ten exposures at 14 ppm mean absolute liver weights were increased compared to controls. White blood cell counts were elevated and lipid like cytoplasmic inclusions were found in hepatocytes and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. Following the two-week recovery period mean organ weights were unremarkable, clinical pathology was normal and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules.<br><br>**Study II**<br>All animals survived until their intended sacrifice. After the 10th exposure, rats from the 420 ppm group exhibited increased mean absolute and relative liver weights compared to controls. A slight loss of cytoplasmic basophilia in hepatocytes and eosinophilic cytoplasmic inclusions in cells of the renal proximal tubule were noted. Lipid like cytoplasmic inclusions were found in hepatocytes, however the same incidence of inclusions was found in the control. Microscopic evaluations were normal following recovery.<br><br>At 42 and 4.2 ppm, lipid like cytoplasmic inclusions were found in hepatocytes, |

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| | however the same incidence of inclusions was found in the control. Microscopic evaluations were normal following recovery. |
| ***Conclusions*** | Under the conditions of this study inhalation exposure to this test material resulted in an elevation of mean liver weights at 14 ppm and greater. Lipid like cytoplasmic inclusions were found in hepatocytes at all concentrations tested however since unrestrained, nonfasted rats did not exhibit a similar finding the inclusion bodies were considered a physiological response to restraint and were not considered exposure related. At 14 ppm and greater the test material exposures were associated with eosinophilic cytoplasmic inclusions in cells of the renal proximal tubule. This is a common finding in male CD rats and was attributed to protein absorption from the glomerular filtrate. This finding was less severe after recovery and was not considered biologically significant at concentrations of 42 ppm or less. A treatment related polycythemia was observed at 150. The Study Director concluded that the no observed effect level was 42 ppm. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code) Restriction due to the fact that this summary was prepared based on a study abstract and poster presentation. Individual data were not available. |
| ***References*** | The Toxicologist (7) 202; 1987 and poster presented at 1987 Annual Society of Toxicology Meeting |
| ***Other*** | Updated: 6/10/2003 |
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**Robust Summary 18-Repeat Dose-2**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| **Method** | |
| Method/Guideline followed | Federal Register, Volume 43, Number 163 (163.82-2, Subchronic 21 Day Dermal Toxicity Study) |
| Test Type | 21-day dermal toxicity study in rabbits |
| GLP (Y/N) | Not Specified |
| Year (Study Performed) | 1981 |
| Species | Rabbit |
| Strain | Albino White (approximately 2-2.6 kg in body weight at initiation) |
| Route of administration | Dermal, 5 days/week, to the clipped, abraded and unabraded, dorsal surface. |
| Duration of test | 15 days of treatment |
| Doses/concentration levels | 0, 50 and 500 mg/kg |
| Vehicle control | No |
| Sex | Males and females |
| Frequency of treatment | Once/day, 5 days/week for a total of 15 doses. |
| Control and treatment groups | Three intact and three abraded male and female rabbits in the control group and in both treated groups. An untreated control group was included in the study. |
| Post exposure observation period | None |
| Statistical methods | Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included ANOVA with a Newman Keuls test. |
| Remarks field for test conditions | The test material was applied to the clipped, abraded or unabraded dorsal surface of the rabbits for 5 days/week for 15 days. Elizabethan collars were used to prevent ingestion. The hair was clipped and shaved from each animal as necessary. The exposed skin of half of the animals was abraded once/week throughout the study. The test material was applied over the clipped area and covered with gauze patches secured in place with surgical hypoallergenic adhesive tape. The trunk of each animal was then wrapped with an impervious material held in place with an elastic bandage. Control animals were handled in an identical manner. After 6 hours the treated areas were wiped gently with corn oil. Clinical observations were made daily. Dermal responses were evaluated daily on dosing days approximately one hour after the completion of the exposure period. Body weight was recorded twice weekly during treatment. Food consumption was estimated every 3 to 4 days during the study. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. Microscopic examinations were conducted for all animals. The following tissues were evaluated: treated and untreated skin, gross lesions, brain, heart, liver, spleen, kidneys, urinary bladder, ovaries, testes, adrenals, thyroid, stomach, mesentery, small and large |

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| | intestine and cecum. |
| ***Results*** | |
| Remarks | Two animals died prior to study termination. These included one high dose female (on day 5) and one low dose male (on day 21). The low dose death was attributed to pneumonia. The cause of the high dose death was not determined. All of the remaining control and treated animals survived the duration of the study.<br><br>Dermal irritation was observed in both test material treated groups. Erythema and eschar formation and edema ranging from well defined to severe were observed in males and females at both dose levels at both intact and abraded dose sites. Findings in the high dose males were more severe than in the low dose males. A dose response was not clearly evident in the females. No significant dermal findings were observed microscopically.<br><br>There were no consistent differences exhibited in mean body weight between the treated and control animals. Food consumption was generally unremarkable in all groups throughout the study. The clinical laboratory, organ weight and microscopic data were also generally unremarkable.<br><br>Based on the in life dermal findings observed in the low dose males and females, a no observed adverse effect level for local effects was established. |
| ***Conclusions*** | Based upon systemic toxicity, a NOEL of >500 mg/kg was established for this study. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code) Restriction due to the lack of correlation between in life dermal findings and microscopic findings of the skin. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 12/2/2003 |
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**Robust Summary 18-Repeat Dose-3**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2-ethylhexyl ester |
| Remarks | 100% |
| **Method** | |
| Method/Guideline followed | Japanese Guidelines for Screening Toxicity Testing Chemicals (Notifications Kanpogyo No. 39, Yakuhatsu No. 229 Kikyoku No. 85 (1984) |
| Test Type | 28-day oral toxicity study in rats |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Species | Rat |
| Strain | SD[Crj: CD(SD), SPF], 4 weeks of age at receipt |
| Route of administration | Oral gavage (syringe and dosing tube) |
| Duration of test | 28 days of treatment plus 14 days of recovery |
| Doses/concentration levels (dose volume) | 0, 20, 100 and 500 mg/kg/day (10 ml/kg) |
| Vehicle | 0.5% Tween 80 |
| Sex | Males and females |
| Exposure period | 28-day treatment duration |
| Frequency of treatment | 7 days/week |
| Number of animals/sex/group | 6 rats/sex/group for 28 day sacrifice<br>6 rats/sex in control and high dose for 14 day recovery period |
| Post exposure observation period | 14 days in control and high dose groups |
| Chemical Analysis | Chemical analysis of dosing solutions was conducted by the sponsor and confirmed dosing suspensions were stable for 10 days. |
| Statistical methods | Student's t-test, Welch's t-test, Armitage's chi square test. |
| Dose rangefinding study | Yes (acute study and 10 day toxicity study) |
| Remarks field for test conditions | Single oral doses were administered for 28 consecutive days using a gavage needle. Clinical observations were performed daily. Body weights were recorded prior to the first dose and weekly thereafter. Food consumption was measured weekly. Hematology, clinical chemistry and urinalysis determinations were conducted prior to the 28 day and recovery sacrifices for all survivors. Macroscopic examinations were performed on all animals. The brain, liver, kidney, adrenal, testis and ovary were weighed. A range of tissues was examined microscopically. These included the heart, liver, spleen, kidney and adrenals from all control and high dose animals, the kidney from all low and mid dose animals sacrificed at 28 days and gross lesions from all groups. The kidneys were examined for all animals at recovery. |
| ***Results*** | |
| Remarks | All of the treated animals survived the duration of the study. Post dosing salivation was observed in the high dose males and females |

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| | during the second week of study and thereafter. Predosing salivation was also observed in the high dose females. Salivation was not observed during recovery.<br><br>A statistically significant decrease was observed in the mean body weight of the high dose females during the last week of treatment and during the first week of recovery. A statistically significant decrease was observed in the mean food consumption of the high dose females during the last week of treatment but not during recovery.<br><br>Following 28 days of treatment the high dose males exhibited a statistically significant increase in mean platelet number. No other treatment related changes were observed in the hematology data. The high dose females exhibited a significant increase in urea nitrogen and a significant decrease in chloride following 28 days of treatment but not following recovery.<br><br>A number of alterations were observed in the urinalysis data of mid and high dose 28 day sacrifice animals, these included: a significantly acidic urinary pH in high dose males and females; a significant increase in protein, ketone bodies, urobilinogen, volume, potassium and chloride in high dose males and females; a significant increase in ketone bodies in the mid dose females; and a significant increase of epithelial cells in the urine sediment of the mid and high dose females. In the high dose recovery females significant decreases in sodium, potassium and chloride were observed.<br><br>Following 28 days of treatment significant increases were observed in the absolute and relative liver and kidney weights of the high dose males and in the relative liver and kidney weights of the high dose females. Increased relative adrenal weights were also observed in the high dose females. Following recovery relative kidney weights were increased in the high dose males.<br><br>At the 28 day necropsy enlarged livers were observed in the mid dose males and high dose males and females. Enlargement of the kidneys was observed in the high dose males. Following recovery enlarged kidney was observed in one high dose male.<br><br>Microscopic changes observed in the kidney of the mid and high dose males included the appearance of hyaline droplets in the proximal tubular epithelium and regenerative changes of the renal tubules. The regenerative change was also observed in one high dose recovery male. None of these effects were observed in the females. There were no significant histological effects in the liver. |

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| ***Conclusions*** | The Study Director concluded that the no observed effect level for systemic toxicity was 20 mg/kg/day. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 10/17/2003 |
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## 4.4 Toxicity to Reproduction

**Robust Summary 18 – Repro – 1**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 27247-96-7 |
| Chemical Name | Nitric acid, 2 ethylhexyl ester |
| **Method** | |
| Method/Guideline | OECD Guidelines 421, EPA Guideline OPPTS 870.3550 |
| Test Type | Reproduction/developmental screening study in rats |
| GLP (Y/N) | Y |
| Year (Study Performed) | 2006 |
| Species | Rat |
| Strain | Sprague-Dawley CD, 11 weeks of age at initiation of treatment |
| Route of administration | Orally by gastric intubation |
| Duration of test | F0 males: 15 day premating period plus mating and postmating periods (at least 4 weeks total).<br>F0 female: 15 day premating period through day 5 of lactation. |
| Dose levels | 0, 20, 100 and 500 mg/kg/day |
| Vehicle control | 0.5% tween 80 in purified water |
| Dose volume | 10 mL/kg |
| Sex | Males and females |
| Frequency of treatment | Once/day, 7 days/week |
| Analytical confirmation of concentration. | Homogeneity, stability and dose concentration (weeks 1, 3 and 6) confirmation. |
| Control and treatment groups | 10/sex/group |
| Post exposure recovery period | None |
| Mating ratio | One male to one female |
| Duration of mating period | Up to 14 days with the same male |
| Statistical methods | Anova, Dunnett's, Fisher's Exact as appropriate. |
| Dose range finding study | A prior 28-day study was conducted. |

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| Remarks field for test conditions | Viability and Toxicity: Twice daily<br>Clinical Observations: At lease once daily throughout the study.<br>Body Weights: F0 males: pretest, day 1 of treatment, weekly through termination.<br>F0 females: pretest, weekly during premating and mating; gestation days 0, 7, 14 and 20; females with litters weighed on lactation days 1 and 5.<br>Food Consumption: Pretest and weekly during treatment period.<br>F0 males: pretest, weekly throughout the study except during mating.<br>F0 females: pretest, weekly during premating, days 0-7, 7-10, 10-14, 14-17 and 17-20 of gestation and days 1-5 of lactation for females with litters.<br>Macroscopic Examinations: Performed on all animals.<br>Pup/Litter Examinations: Litters observed as soon as possible after delivery for number of live and dead pups and pup abnormalities. Thereafter litters observed daily for dead pups and/or obvious irregularities.<br>Litter Size: Number of live and dead pups recorded on days 0 and 4 of lactation.<br>Individual Pup Body Weights: Pup weights recorded on days 1 and 5 of lactation.<br>Necropsy: F0 all animals were sacrificed by exposure to carbon dioxide followed by exsanguinations (males) or cervical dislocation (females)<br>(males: after the end of the mating period; females: day 6 *post-partum;*<br>females which had not delivered by day 25 *post-coitum*: day 25 or day 26 *post-coitum*; mothers with litter dying entirely: as appropriate; surviving pups: day 6 *post-partum).*<br>Macroscopic Examinations: A macroscopic *post-mortem* examination of the principal thoracic and abdominal organs (with particular attention to the reproductive organs) was performed on all parent animals including<br>any which were sacrificed prematurely. The number of implantation sites and *corpora lutea* was recorded, for all the females, whenever possible. In the females which were apparently non-pregnant, the presence of implantation scars on the uterus was checked using ammonium sulphide staining technique.<br>Pups found dead and pups sacrificed on day 6 post-partum (or shortly hereafter) were carefully examined externally for gross external abnormalities, and a macroscopic examination was performed. Tissues were not retained.<br>Microscopic Examinations: Select tissues were examined microscopically for all control and high dose F0 animals. Gross lesions and tissue masses were examined microscopically for all F0 animals. |

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| ***Results*** | Analysis of dosing solutions confirmed that the preparations were homogeneous and stable for their intended period of use and that they were at the appropriate concentrations.<br><br>There were no animals prematurely sacrificed for reasons of poor clinical condition during the study. All animals given 500 mg/kg/day had excessive salivation throughout the study. Hypoactivity, half-closed eyes and loud breathing were also observed sporadically in a few males and females. The majority of the females given 100 mg/kg/day had excessive salivation during the premating and gestation periods and a few males had excessive salivation during the premating period, which may have been related to the taste of the test formulations. One female at 100 mg/kg/day had hypoactivity and half-closed eyes on day 12 of dosing. There were no clinical signs at 20 mg/kg/day.<br><br>Male and female groups at all dose-levels gained less weight than the controls during the premating period in a dose-related manner achieving statistical significance at 500 mg/kg/day. Females given 500 mg/kg/day continued to gain less weight during gestation, however all groups had approximately comparable weight gains to the controls during lactation. Females given 500 mg/kg/day consumed less food than the controls throughout the study.<br><br>All pairs mated and the mean number of days taken to mate was comparable with the controls for all groups. There were no effects on the numbers of *corpora lutea* or implantations. The mean numbers of pups born per litter were comparable with the controls at all dose-levels.<br><br>At 500 mg/kg/day, there was one female with one dead fetus and nine implantation scars in the uterine horns and one female which delivered a litter of small pups, all of which were dead by day 3 post-partum.<br>There were no treatment-related pup clinical signs or necropsy findings.<br>There were no treatment-related findings observed during the microscopic examination.<br><br>Based on the experimental conditions of this study, the No Observed Adverse Effect Level (NOAEL) for parental toxicity was considered to be 20 mg/kg/day, and the NOAEL for toxic effects on reproductive performance and on pups was 100 mg/kg/day. |
| ***Conclusions*** | The No Observed Adverse Effect Level (NOAEL) for parental toxicity was considered to be 20 mg/kg/day, the NOAEL for toxic effect on reproductive performance and on pups was 100 mg/kg/day. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Davies, R. "Reproduction/Developmental Toxicity Screening Test |

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| | by Oral Route (Gavage) in Rats." CIT Safety & Health Research Laboratories Number 29497 RSR. 28 June 2006. |
| ***Other*** | Updated: 8/25/06 |
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